

030

Comparison of alignment variations

Proposed variations

Version 1.0 → A only  
 Version 2.0 → B only  
 Version 3.0 → A, B no overlap  
 Version 4.0 → no A, B

Version 5.0  
 megas A83335.11  
 A83335.10

aligned at 100 pps / sec  
 50.2 each mixed  
 ↓  
 3/4 slow coal  
 1/4

Version 6.0 7.28 aligned (100 pps)  
 2.28 42% out  
 7.28 4.28  
 1.528 10% out  
 1.5 12.5 100% A/B  
 1.528 1.528  
 1.528  
 1.528 1.528  
 1.528 1.528



*John A. Sease*  
*Mike Sease*

## Protease Procedures

Name	Description	Register	Cell vector	Transform	Strains
PGN01	mouse polyubiquitin 1 (UBI1)	GLB	pGEM004	Com	GSA
PGN02	mouse polyubiquitin 1	GLB-Adh	pGEM002	Com	G38
PGN03	mouse 22 kD ochratoxin	GLB-Adh	pGEM003	Com	IRAD
PGN04	mouse UBI1 no histone elements (HSE-UBI1)	GLB-Adh	pGEM001	Com	G38
PGN05	mouse UBI1 no 3' HSE-Ubi1	GLB-Adh	pGEM007	Com	G38
PGN06	mouse UBI1 no 5' HSE-Ubi1	GLB-Adh	pGEM006	Com	G38
PGN07	mouse UBI1 no HSE-Ubi1	GLB-Adh	pGEM008	Com	G38
PGN08	mouse UBI1 replace HSE with 3' P1 seed specific element (UBI3)	GLB-Adh	pGEM009	Com	G38
PGN09	leucine polyubiquitin 1	GLB-Adh	pGEM010	Com	G38
PGN10	leucine polyubiquitin 1	GLB-Adh	pGEM011	Com	G38
PGN11	leucine polyubiquitin 1	GLB-Adh	pGEM012	Com	G38
PGN12	mouse polyubiquitin-1 (GST1)	GLB-Adh	pGEM013	Com	G38
PGN13	synthetic promoter RpyD with 35S enhancer & fused with mouse Adh-1 3'UTR	GLB-Adh	pGEM014	Com	G38
PGN14	synthetic promoter RpyD with 35S enhancer & fused with mouse Adh-1 3'UTR	GLB-Adh	pGEM015	Com	G38
PGN15	mouse RpyD	GLB-Adh	pGEM016	Com	G38
PGN16	mouse RpyD fused with mouse Adh-1 3'UTR	GLB-Adh	pGEM017	Com	G38
PGN17	modified version of Agro intronless synthase (AgroMAS)	GLB-Adh	pGEM018	Com	G38
PGN18	bean phytoalexin	GLB-Adh	pGEM019	Com	G38
PGN19	mouse UBI1 no 5' HSE with 0.8 kb extended seq of 3' end (beyond p4)	GLB-Adh	pGEM020	Com	G38
PGN20	mouse UBI1 no 5' HSE with 0.8 kb extended seq of 3' end (beyond p4)	GLB-Adh	pGEM021	Com	G38
PGN21	mouse UBI1 no 5' HSE with 0.8 kb extended seq of 3' end (beyond p4)	GLB-Adh	pGEM022	Com	G38
PGN22	mouse UBI1 no 5' HSE with 0.8 kb extended seq of 3' end (beyond p4)	GLB-Adh	pGEM023	Com	G38
PGN23	mouse polyubiquitin 2	GLB-Adh	pGEM024	Com	G38

## GIBCO BRL Custom Primers

### Certificate of Analysis

**Primer 1:**

Primer Name: UBI HSP VER. 1A

Researcher:

Primer Number: A8333C10 (C10)

Primer Length: 69

Sequence (5' to 3'): PAG ACG GCA CGG CAT CTC TGT CGC TGC CTC CAC CGT TGG ACT TGC TCC GCT  
GTC GCC ATC CAG AAA TMolecular Weight  $\mu\text{g}/\mu\text{mole}$ : 21299.2

Millimolar Extinction Coefficient: 678.9

Purity: Desalted

Tm (1 M Na<sup>+</sup>): 96Tm (50 mM Na<sup>+</sup>): 76

% GC: 80

Notes:

 $\mu\text{g per OD}$ : 31.3

nmoles per OD: 1.4

OD's: 39.3

 $\mu\text{g's}$ : 1234

nmoles: 67

Coupling Eff.: 99%

~52%  
~1"**Primer 2:**

Primer Name: UBI HSP VER.1B

Researcher:

Primer Number: A8333C11 (C11)

Primer Length: 67

Sequence (5' to 3'): PTT TCT GGA TGC CGA CAG CCG AGC AAG TCC AAC GGT GGA GGC AGC GAC AGA  
GAT GCC GTG CCG TCT GCMolecular Weight  $\mu\text{g}/\mu\text{mole}$ : 21897.4

Millimolar Extinction Coefficient: 732.9

Purity: Desalted

Tm (1 M Na<sup>+</sup>): 97Tm (50 mM Na<sup>+</sup>): 78

% GC: 62

Notes:

 $\mu\text{g per OD}$ : 29.8

nmoles per OD: 1.3

OD's: 10.7

 $\mu\text{g's}$ : 319

nmoles: 14

Coupling Eff.: 98%

VER 1A

57 nmoles

570  $\mu\text{L}$   $\rightarrow$  100  $\mu\text{mol}/\mu\text{L}$ 

14 nmoles

140  $\mu\text{L}$   $\rightarrow$  200  $\mu\text{mol}/\mu\text{L}$ 

\* See Note about Quantities in  
Supporting Information.

LIFE  TECHNOLOGIES.

# GIBCO BRL Custom Primers Certificate of Analysis

**Primer 1:**

Primer Name: UBI HSPA VER.2A

Researcher:

Primer Number: D0373887 (B07)

Primer Length: 81

Sequence (5' to 3'): P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CGG TGT CGA CCA CCG  
TTG GAC TTG CTC CGC TGT CCG CAT CCA GAA ATMolecular Weight  $\mu\text{g}/\mu\text{mole}$ : 25105.2

Millimolar Extinction Coefficient: 824.3

 $\mu\text{g}$  per OD: 31.5

nmoles per OD: 1.2

Purity: Desalt

OD's: 90.0

Tm (1 M Na+): 98

 $\mu\text{g/s}^*$ : 2850

Tm (50 mM Na+): 77

nmoles: 108

% GC: 61

Coupling Eff.: 98%

Notes:

**Primer 2:**

Primer Name: UBI HSPB VER.2B

Researcher:

Primer Number: D0373888 (B08)

Primer Length: 82

Sequence (5' to 3'): P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG TCG AGA GGG GTC  
CAG AGG CAG CGA CAG AGA TGG CGT GCG GTC TGCMolecular Weight  $\mu\text{g}/\mu\text{mole}$ : 26872.4

Millimolar Extinction Coefficient: 902.2

 $\mu\text{g}$  per OD: 29.7

nmoles per OD: 1.1

Purity: Desalt

OD's: 77.8

Tm (1 M Na+): 99

 $\mu\text{g/s}^*$ : 2294

Tm (50 mM Na+): 78

nmoles: 86

% GC: 63

Coupling Eff.: 98%

Notes:

**Primer 3:**

Primer Name: UBI HSPA VER.3A

Researcher:

Primer Number: D0372809 (B09)

Primer Length: 81

Sequence (5' to 3'): P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTC GAG AGT TCC GCT CCA CCG  
TTG GAC TTG CTC CGC TGT CCG CAT CCA GAA ATMolecular Weight  $\mu\text{g}/\mu\text{mole}$ : 25160.2

Millimolar Extinction Coefficient: 830.6

 $\mu\text{g}$  per OD: 31.5

nmoles per OD: 1.2

Purity: Desalt

OD's: 88.7

Tm (1 M Na+): 98

 $\mu\text{g/s}^*$ : 2783

Tm (50 mM Na+): 76

nmoles: 106

% GC: 60

Coupling Eff.: 98%

Notes:

\* See Note about Quantities in  
Supporting Information.

**LIFE TECHNOLOGIES.**

# GIBCO BRL Custom Primers Certificate of Analysis

**Primer 4:**

Primer Name: UBI HSPB VER.3B

Researcher:

Sequence (5' to 3'): P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CCG TGG AGC GGA ACT CTC  
GAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Primer Number: D0373B10 (B10)

Primer Length: 82

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 26518.4

Millimolar Extinction Coefficient: 901.3

 $\mu\text{g}$  per OD: 29.7

nmoles per OD: 1.1

Purity: Desalt

Tm (1 M Na<sup>+</sup>): 99Tm (50 mM Na<sup>+</sup>): 77

% GC: 62

Notes:

OD's: 81.2

 $\mu\text{g/s}$ : 2478

nmoles: 92

Coupling Eff.: 98%

930  $\mu\text{g}$   $\rightarrow$  100  $\mu\text{g}$ **Primer 5:**

Primer Name: UBI HSPA VER.4A

Researcher:

Sequence (5' to 3'): P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CCG TCT CGA CTC GAG  
AGT TCC GCT CCA CCG TTG GAC TTG CTC CGC TGT CCG CAT CCA GAA AT

Primer Number: D0373B11 (B11)

Primer Length: 86

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 30988.2

Millimolar Extinction Coefficient: 978.3

 $\mu\text{g}$  per OD: 31.7

nmoles per OD: 1.0

Purity: Desalt

Tm (1 M Na<sup>+</sup>): 100Tm (50 mM Na<sup>+</sup>): 78

% GC: 61

Notes:

OD's: 89.3

 $\mu\text{g/s}$ : 2833

nmoles: 91

Coupling Eff.: 98%

930  $\mu\text{g}$   $\rightarrow$  100  $\mu\text{g}$ **Primer 6:**

Primer Name: UBI HSPB VER.4B

Researcher:

Sequence (5' to 3'): P-T TTT TGG ATG CCG ACA GCG GAG CAA GTC CAA CCG TGG AGC GGA ACT CTC  
GAG TCG AGA CCG GTC CAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Primer Number: D0373B12 (B12)

Primer Length: 87

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 31781.4

Millimolar Extinction Coefficient: 1070.6

 $\mu\text{g}$  per OD: 29.6

nmoles per OD: 0.9

Purity: Desalt

Tm (1 M Na<sup>+</sup>): 100Tm (50 mM Na<sup>+</sup>): 79

% GC: 62

Notes:

OD's: 97.1

 $\mu\text{g/s}$ : 2883

nmoles: 90

Coupling Eff.: 98%

930  $\mu\text{g}$   $\rightarrow$  100  $\mu\text{g}$ \* See Note about Quantities in  
Supporting Information.

9-007

Do Nucleobond prep of 5596, 5597,  
4216, 4217, 4218 and 4219

Digest 5596 and 5597 w/ EcoRI as a check  
make sure smaller frag. is ~ 2kb.

Digest 4216, 4217, 4218, 4219 w/ Bgl II/Sal I  
to check that the 168bp frag. is generated.

Digest 4216 w/ Bgl II/Xba I to use as  
accepting vector for ubiquitin versions 1-4.  
Gel isolate on 10% agarose

Digest 5596 w/ Nhe I/Not I to isolate insert  
(~ 1.6 kb) L4-BASS. Not I and Nhe I  
Gel isolate on agarose cut in different buffer  
cut w/ Not I 1st. Debris  
and EtOH ppt.  
cut w/ Nhe I

Mini-prep on BASS:NA #5 5648-5665  
Digest w/ EcoRI/Pst I. Cut 1.8 also  
Run on 10% agarose

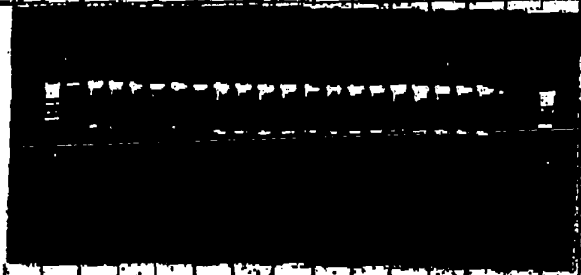
→ Anneal Ubiquitin versions 1, 2, 3, and 4  
aligns together  
Heat to 95°C for 5 min then stick on ice  
Check these on a 10% agarose gel

Run pre-cut 3270 Nhe I/Not I on agarose  
gel to check it out. ~~rather simple~~

13-004

Check the variation w/ a 113/110T digest (first 5 of each)

1. 1 Kb ladder
- 2-6. Lanes 1 7543 - 7597
- 7-11. Lanes 2 7561 - 7565
- 12-16. Lanes 3 7579 - 7583
- 17-21. Lanes 4 7597 - 7601
22. ~~5000~~ NL/110T 7662 vector
23. 6939 NL/110T Spg
24. 1 Kb ladder 8



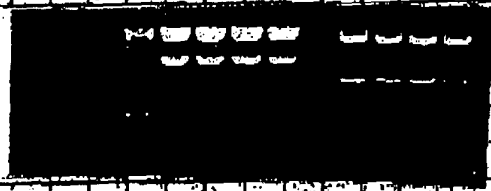
All look fine. Send these for sequencing.

Sequencing data shows 7368 to be correct for G43/R2P.

Make Avidin/R2P using 7368 as standard.

Digest 7424 (Avidin/3720) and 7368 (G43/R2P) with BamHI/HaeIII.

- Gel: 1. 1 Kb ladder
- 2-5. 7368 (G43/R2P) BamHI
6. Spg
- 7-10. 7424 (Avidin) BamHI



Isolate large vector frag from G43/R2P and the smaller insert band from Avidin.

Katherine Harper



Gus Assay

PURPOSE: TO QUANTITATE THE AMOUNT OF GUS IN CORN SEED

## MATERIALS:

REACTION PLATE - COSTAR EIA/RIA

READING PLATE - NUNC FLUORENCE POLYSOEP

MU - 4 METHYLLUMBELLIFERONE (SIGMA H-1508)

MUG - 4 METHYLLUMBELLIFERONE B GLUCURONIDE (SIGMA H-9130)

MICROPLATE

FLUORESCENCE MICROPLATE READER

PROCEDURE: USE PROTOCOL FOUND ON PAGE # 57 OF THIS NOTEBOOK (#58)

RESULTS: DATA FOUND BELOW (BASED ON 20-NEW READINGS)

SAMPLE #	%TSP	SAMPLE #	%TSP
05E12020-4	0.088	05E 05230-1	0.087
-5	ND	-2	0.54
05D 01120-1	ND	-3	0.61
-2	ND	-4	0.16
-3	ND	-5	0.06
-4	ND	11 0828-1	0.021
-5	ND	-2	0.002
05E 15070-4	0.28	-3	0.027
11 05050-1	0.17	-4	ND
-2	0.015	-5	0.001
-3	0.010	11 07050-1	0.3
-4	0.174	-2	0.089
-5	0.010	-3	0.27
11 05090-1	0.043	-4	0.013
-2	0.014	-5	0.43
-3	0.001		
-4	0.004		
-5	0.004		
05E 01010-1	0.026		
-2	0.010		
-3	0.009		
-4	0.60		
-5	0.48		

Investigator:

Book # 58

Chris Brook Date:

Witness:

Elizabeth Wilcox Date:



GUS ASSAY

SEE PURPOSE, MATERIALS, &amp; PROCEDURE BELOW.

**Purpose:** To quantify the amount of GUS in corn seed extracts.**Materials:** Reaction Plate-Costar 96WELLS, non-adhesive bottom 96-well flat bottom plate  
Reading Plate-Beckman Coulter 550-well black plate  
MUG 4-methylumbelliferyl-β-D-glucuronide (Sigma M-2500)  
MUGO 4-methylumbelliferyl-β-D-glucuronide (Sigma M-2500)  
Microplate Fluorometer (Spectra-Fluorometer)**Reagents:** 1.0 M Tris-HCl, 20 mM sodium phosphate, pH 7.0, 1 mM EDTA, 10 mM MgCl<sub>2</sub>Note: 20 mM sodium phosphate is made by mixing 97 ml of Stock A (0.2 M Na<sub>2</sub>HPO<sub>4</sub>, 27.5 g/L) with 1.5 ml of Stock B (0.2 M NaH<sub>2</sub>PO<sub>4</sub>, 27.5 g/L) and bringing to a final volume of 1.0 L with dH<sub>2</sub>O.

Also note that the 10 mM EDTA should be added to an aliquot of the Tris buffer from daily, enough for that day's experiment.

Stock Buffers: 0.2 M Na<sub>2</sub>HPO<sub>4</sub> (27.5 g/L)  
1 mM MUG standard stock: 4.0 mg MUG in 25 ml dH<sub>2</sub>O (make fresh daily)  
20 mM MUGO standard stock: 7 mg MUGO in 1.0 ml 95% ethanol (make fresh daily)**Procedure:** Corn seed extracts should already be prepared and assayed for total protein according to standard procedures.

In a reaction plate, replicate up to 96 spots of seed extracts in a final volume of 100 μl Tris buffer. Generally samples can be assayed with 1 μg total protein. Samples should be assayed in triplicate.

Add standard curve to triplicate wells as follows:

10 μl of 1 mM MUG standard stock is diluted with 90 μl Tris buffer.  
20 μl of this 1:10 dilution is further diluted with 80 μl Tris buffer to give a 1:100 dilution.  
0 mM MUG standard  
1000 μM MUG standard  
10,000 μM MUG standard  
100,000 μM MUG standard  
100 μl Tris buffer / well  
12.5 μl of the 1:100 dilution = 0.1 μM MUG / well  
12.5 μl of the 1:10 dilution = 0.2 μM MUG / well  
12.5 μl of the 1 mM MUG stock = 0.2 μM MUG / well

CB

Prepare the reaction plates by pipetting 175 μl of Tris buffer into each well of the plate. You will need a separate plate for each time point sampled. Generally we take readings at 0, 15, 30 and 60 minutes.

Then the 20 mM MUGO substrate stock is 2 mM with Tris buffer. Add 25 μl of 2 mM MUGO to every well including both standard and sample wells and mix to start the reaction. Immediately after adding the MUGO, pipette 25 μl of solution from the reaction plate into a prepared reading plate. Then the reaction plate is at 37 °C until the next time point. At each subsequent time point, pipette 25 μl of solution from the reaction plate into a prepared reading plate.

Reaction is stable for several hours once it has been stopped. Note that stopping the reaction is essential for fluorescence detection.

Plates are read at 360 nm excitation wavelength and 460 nm emission wavelength.

The unknown samples are read against the standard curve to add MUG and the amount of GUS in the sample is calculated as follows:

Average MUG for each sample (from Value Column) / unknown reaction provided = MUG / ml = 60 min / ml = MUG / ml. Note that if there is a 100 μl in the 96 well plate reading of the average MUG, that value must be subtracted from the average MUG of each subsequent reading. This value is then converted for the amount of protein added to the sample by dividing by the total protein added to give μM MUG / μg protein. This value is converted to %TSP by multiplying by 1.0E+10 which is a conversion factor determined while at Fluor.

A Quality Control sample (a known amount of GUS) is assayed into several rows and columns to give an overall average to determine repeatability of quantification.

CB

## RESULTS: DATA FOUND BELOW. (10-MIN READINGS)

Sample #	%TSP	Sample #	%TSP	Sample #	%TSP
GSC 01040-1	0.0	GSC 01110-1	0.6 0.06	GSC 01060-1	0.0
-2	0.4 0.04	-2	0.4 0.04	-2	0.0
-3	0.6 0.06	-3	0.0	-3	0.0
-4	0.5 0.05	-4	0.4 0.04	-4	0.0
-5	0.4 0.04	-5	0.4 0.04	-5	4.8 0.5
GSD 02130-1	tot 0.1	GSC 01070-1	4.2 0.4	GSC 01130-1	8.4 0.8
-2	0.7 0.07	-2	2.7 0.3	-2	0.1 0.01
-3	0.9 0.1	-3	3.7 0.3	-3	8.6 0.9
-4	0.0	-4	5.2 0.5	-4	5.0 0.5
-5	0.8 0.1	-5	0.01 0.001	-5	0.7 0.07
GSC 01020-1	0.0	GSC 01040-1	0.1 0.01	GSC 01110-1	0.0
-2	0.0	-2	5.1 0.5	-2	9.2 0.9
-3	0.12 0.01	-3	0.3 0.03	-3	0.0
-4	0.0	-4	0.3 0.03	-4	0.0
-5	0.2 0.02	-5	0.04 0.004	-5	9.6 0.7
GSC 01030-1	0.0				
-2	4.0 0.4				
-3	4.2 0.4				
-4	0.5 0.05				
-5	9.5 0.8				

Investigator:  
Investigator:

Block # 67

Chris Bivola  
Date:

Witness:

Elizabeth Wilfong  
Date: